

METHOD AND APPARATUS FOR PRETREATMENT OF TISSUE SLIDES

This application claims the benefit of U.S. Provisional Application No. 60/435,601, filed December 20, 2002.

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BACKGROUND

The present invention relates to a method and an apparatus for automatic staining at least one tissue sample accommodated on a slide by applying reagents in a sequence according to a staining protocol, wherein at least one slide may be provided in a slide rack.

This application relates to the field of sample processing systems and methods of processing samples. The present invention may be directed to the automated processing, treatment, or even staining of samples arranged on carriers, such as microscope slides, and in some embodiments, directed to the continuous or batch processing of samples and carriers, as well as washing elements of a sampling system. Embodiments may further relate to control systems for sample processing and data acquisition, data maintenance, and data retrieval for sample processing. Applications to which the present invention may especially relate include immunohistochemistry, in-situ hybridization, fluorescent in-situ hybridization, special staining, and microarrays, as well as potentially other chemical and biological applications.

Sample processing in immunohistochemical (IHC) applications and in other chemical and biological analyses may require one or a number of various processing sequences or protocols as part of an analysis of one or more samples. The sample processing sequences or protocols may be defined by the individual or organization requesting an analysis, such as a pathologist or histologist of a hospital, and may be further defined by the dictates of a particular analysis to be performed.

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In preparation for sample analysis, a biological sample may be acquired by known sample acquisition techniques and may comprise, for example in IHC applications,

tissues generally or even in some applications one or a plurality of isolated cells, such as in microarray samples, and may be presented on a sample carrier such as a microscope slide. Furthermore, the sample may be presented on the carrier variously and potentially in some form of preservation. As one example, a sample
5 such as a layer or slice of skin may be preserved in formaldehyde and presented on a carrier with one or more paraffin or other chemical layers overlying the sample.

Immunologic applications, for example, may require processing sequences or protocols that comprise steps such as deparaffinization, target retrieval, and staining,
10 especially for in-situ hybridization (ISH) techniques. Previously, in some applications, these steps may have been performed manually, potentially creating a time-intensive protocol and necessitating personnel to be actively involved in the sample processing. Attempts have been made to automate sample processing to address the need for expedient sample processing and a less manually burdensome
15 operation. However, such previous efforts may not have fully addressed the needs for an automated sample processing system. Previous efforts to automate sample processing may be deficient in several aspects that prevent more robust automated sample processing, such as: the lack of sufficient computer control and monitoring of sample processing; the lack of information sharing for processing protocol and
20 processing status, especially for individual samples; the lack of diagnostic capabilities; and the lack of real-time or adaptive capabilities for multiple sample batch processing.

Past efforts at automated sample processing for samples presented on carriers such as
25 slides, such as US Patent No. 6,352,861 and US Patent No. 5,839,091, have not afforded the various advantages and other combinations of features as presented herein.

The tissue samples may be prepared before the staining can be performed. The tissue
30 slides may be subjected to a pretreatment process depending on the type of staining process that may be performed on the tissue. This pretreatment could include a deparaffinization or a target retrieval. The preparation of the tissues on the slides may

often be carried out manually in the laboratory before they are loaded into the automatic staining instrument. This pretreatment may include immersing the slide in a buffer or other types of processing liquid for a predetermined amount of time and temperature.

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However, this manual preparation may be cumbersome and the pretreatment may be insufficient, since it may be critical that the amount of time and the temperature of the liquid must be observed very precisely in order to achieve the correct pretreatment result.

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SUMMARY OF INVENTION

It is an object of the invention to provide an automatic pretreatment of the slides in an automatic staining apparatus so that the entire tissue sample processing may be performed in a single automatic apparatus.

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The invention consists of a method of treatment of at least one tissue sample accommodated on a slide in an automated staining apparatus, the method comprising the steps of: providing at least one slide having a tissue sample which may even be in a predetermined slide location, a slide may be in a substantially horizontal position; pivoting at least one slide to a substantially vertical position; immersing a substantially vertically oriented slide into a fluid containment element or even in a dip tank for a processing time which may be predetermined. In embodiments, the slide may be located in a slide rack, a plurality of slides may be provided in a slide rack, and even the slides may be individually pivotable.

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An object may be also achieved by an apparatus for automatic staining at least one tissue sample accommodated on a slide by applying a predetermined amount of reagents in a sequence according to a staining protocol, wherein at least one slide may be provided in a slide rack assembly or even a slide rack, wherein the slide rack assembly may comprise means for pretreatment of the tissue sample on a slide after the slide may be provided in the slide rack assembly or more broadly a pretreatment

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element. Said means for pretreatment of tissue samples may include a fluid containment element or even a dip tank which may be provided in a slide rack assembly; a slide rack holder; and means for pivoting the slides provided in the slide rack holder to a vertical position or more broadly a vertical slide positioner adapted to pivot at least one slide in a vertical position and means for immersing the vertical slide into the dip tank or more broadly a slide immerser element adapted to immerse at least one slide in a fluid containment element.

By pivoting the slides from a horizontal to a vertical position, an automated method and apparatus for carrying out the pretreatment in the automated staining apparatus may be provided. A pivoting of slides may ensure an appropriate orientation of the slides for both the pretreatment and the staining processes.

By the present invention, the preparation of the tissue samples on the slides may be integrated in an automatic staining apparatus, so that a tissue sample once it may be accommodated on a slide can be loaded into a staining apparatus wherein both the pretreatment and the staining protocols may be performed automatically in the apparatus.

In embodiments, a selected processing liquid may be supplied from at least one supply tank into a fluid containment element or even a dip tank. The steps of filling and draining a dip tank may be controlled by a control system of the apparatus which may ensure that the slides are subjected to the appropriate pretreatment steps according to information concerning this in the staining protocol.

In other embodiments, the liquid may be transferred from a supply tank to a transfer tank and onwards to a dip tank. The apparatus may comprise pneumatic means for transferring processing liquid, or more broadly the present invention may include a pneumatic element adapted to transfer liquid from a supply tank to a transfer tank and from the transfer tank to the dip tank and draining liquid or a drain element adapted to remove liquid from the dip tank to the transfer tank and from said transfer tank to a waste collection tank. An advantage achieved by transferring the liquids by a

pneumatic system may be that the at least one transfer tank, the supply tank and the waste tank may be positioned outside the apparatus, and may hereby allow for an increased capacity as there are no dimensional constraints on the tank sizes. Furthermore, in an embodiment of the invention it may be possible to subject the

5 immersed slide to a series of fluids which are sequentially filled and drained from the dip tank, since the system may easily be adapted to contain several supply tanks containing different liquids and similarly also several waste tanks so that it may be possible to sort the waste.

10 In an embodiment, a fluid containment element or even a dip tank may be provided with a heating member for heating the processing liquid contained in a fluid containment element or even a dip tank, and the heating member may advantageously be capable of heating the dip tank content to an elevated temperature of at least 95°C, or even at least 120°C. Hereby, the temperature of the fluid in the dip tank may be

15 heated up to 120°C or even as high as 150°C and kept at this temperature for between 10 to 20 minutes without any sign of boiling. In an embodiment, the heating member may be adapted to heat the fluid to a temperature of 95°C for 40 minutes or more for performing a target retrieval process.

20 According to an embodiment of the invention, the at least one vertical slide may be immersed into the tank by lowering the slide rack holder or even a slide holder and immersing at least one slide into a fluid containment element or even a dip tank after at least one slide may be pivoted to a vertical position. Hereby, a compact dip tank arrangement and slide rack holder lay out may be achieved.

25 The method of treatment of tissue could be a pretreatment of the tissue sample. However, by the invention it may be realized that other types of treatment may be performed on the tissue sample on the slide in a vertical position, such as rinsing the slides. Other processes that may be performed by an apparatus according to the

30 invention include deparaffinization, target retrieval processes, or other processes of the tissue sample.

According to embodiments of the present invention, a fluid containment element or even a dip tank may be an elongated tank having an upper opening slot which may allow vertically oriented slides to be inserted into the container for treatment. Hereby, the volume of a fluid containment element or even a dip tank and thereby the amount of processing fluid needed may be minimized.

In other embodiments, the apparatus may include means for recycling the drained liquid for re-use in a later pre-treatment process of slides or more broadly a liquid recycle element adapted to provide recycled liquid. Hereby, the amount of fluids used for the operation of the apparatus, i.e. a pretreatment and the rinsing of the tissue slides, may be minimized and may result in an easier waste handling and a reduction in costs.

In an embodiment of the invention, a slide rack assembly may be provided in a drawer assembly which may be retracted from the apparatus for loading and unloading of slides. The drawer assembly may cooperate with a fluid containment element or even a dip tank in the drawer receiving means of the apparatus, a dip tank may be capable of simultaneous processing of a plurality of slides accommodated in a plurality of slide holders in the slide rack assembly or even a slide rack. The drawer assembly may provide an apparatus with a great flexibility in use, as slides may be loaded or unloaded from one drawer while the slides in the other drawers may be processed independent thereof. Furthermore, the use of drawers may make it easy to operate an automatic staining system. A fluid containment element or even dip tanks for each of the drawers may be connected to common supply and waste tanks which advantageously may be arranged outside the apparatus.

BRIEF DESCRIPTION OF THE FIGURES

The invention is described with reference to various embodiments with reference to the drawings, in which:

- Fig. 1 is a schematic perspective view of a staining apparatus according to the preferred embodiment of the invention;
- Fig. 2 is a top view of the work area in the staining apparatus shown in fig. 1;
- 5 Fig. 3 is a schematic front view of a drawer assembly including a slide rack assembly and a dip tank in an apparatus according to the invention;
- Fig. 4 is a perspective view of a drawer assembly in a closed position;
- 10 Fig. 5 is the drawer assembly of fig. 4 in an open position;
- Fig. 6 is a perspective top view of a slide holder according to a preferred embodiment of the invention;
- 15 Fig. 7 is a detailed view of the slide holder and the dip tank arranged in a drawer assembly;
- Fig. 8 is a perspective view of a dip tank according to the preferred embodiment
- 20 of the invention;
- Fig. 9 is a front view of the dip tank of fig. 8; and
- Fig. 10 is a fluidic diagram of the handling of processing liquid for the dip tank.
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DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

As mentioned earlier, the present invention includes a variety of aspects, which may be combined in different ways. The following descriptions are provided to list

30 elements and describe some of the embodiments of the present invention. These elements are listed with initial embodiments, however it should be understood that they may be combined in any manner and in any number to create additional

embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described systems, techniques, and applications. Further, this description should further be understood to support and encompass descriptions and claims of all the various
5 embodiments, systems, techniques, methods, devices, and applications with any number of the disclosed elements, with each element alone, and also with any and all various permutations and combinations of all elements in this or any subsequent application.

10 A staining apparatus 1 according to an embodiment of the invention is shown in figures 1 and 2. The staining apparatus 1 may comprise a rectangular frame 4 surrounding a reagent station 2 comprising an array of reagent bottle compartments wherein each compartment a reagent vial 3 may be placed, and a first and second slide
15 sections 5 wherein a number of separate rack assemblies 6 may be placed, and where each rack assembly 6 accommodates a number of microscope slides 7 mounted side by side in a rack assembly 6. In the embodiment shown, each rack may hold up to 8 slides, but the rack may be designed to hold any suitable number of slides. With eight racks arranged side by side, the shown embodiments may hold up to 64 slides 7 each having a sample, e.g. a tissue mounted on the upper side of the slide, so that at least
20 one reagent may be applied from above to the sample on each slide.

A robot arm 20 for moving a probe 10 in X and Y (as well as Z) direction as indicated by the arrows X and Y may be arranged above the frame 4 of the staining apparatus. The robot arm 20 may therefore position the probe 10 above all reagent vials 3 as well
25 as above all the microscope slides 7, and may further operate the probe 10 to aspirate portions of reagent contained in any of the vials 3, to transfer the portion of reagent and apply it to any of the slides 7 in order to provide a selected staining or treatment of the sample on each slide 7. By use of suitable control means e.g. a computer (not shown) having the appropriate software and input data for the purpose, this staining
30 apparatus 1 may be able to automatically staining or treating samples requiring different staining or treatment reagents and processes.

As shown in fig. 1, the probe 10 may be accommodated in a robotic head 22 and may be manipulated by the robot arm 20. The probe 10 may be raised to an upper position (in a Z direction) where it may be clear of the vials 3 underneath the probe 10, but the robot may include means in the robotic head 22 for lowering the probe 10, or more broadly a lowering element, in order to dip the probe tip into the content of a selected reagent vial 3 and to aspirate a selected amount of reagent for the selected staining or treatment process. The robotic head 22 may be also provided with a CCD camera 25 pointing downwards. The camera may be utilized to determine status information of the slides and the reagent bottles and other features of the apparatus in the work area, for example reading a code provided on a reagent container to determine the reagent type and the reagent location within the system. The camera may also determine status of the tissue sample carriers, for example the location of a particular slide, informational indicia, such as a code, that indicate information about the tissue sample presented on the slide or the processing protocol to be performed.

In an embodiment, the staining apparatus 1 of the present invention may further comprise a probe washing station 8 and a reagent mixer 9, and the robot arm 20 may be furthermore arranged to transfer the probe to the washing station 8 as well as to the reagent mixer 9.

The slides 7 in the slide rack assembly 6 may be loaded and unloaded in a horizontal position when the slide rack assembly 6 may be in an upper position, as shown in fig. 3. The slide rack assembly 6 may be arranged in a slide elevator 63 and the slide holder 62 may be adapted to pivot the slide 7 between a horizontal position to a vertical position 7v, when the slide rack 6 may be in an upper position. The slide rack assembly 6 may include a slide rack elevator 63 and may be arranged as a moving part 100a of a drawer assembly 100. In a corresponding stationary part 100b of the drawer assembly 100, a fluid containment element 101 or even a dip tank may be provided.

The system may include eight drawer assemblies, as shown in figure 1. However, it may be realized that any other number may also be provided depending on the design

preferences. Each drawer assembly may include a drawer slide, a slide elevator 63, a slide rack assembly 6 which may include slide temperature control members 64, fluid containment element 101 or even a dip tank, a drip tray 65 for collecting staining fluids and a control means, or more broadly a control element which may include
5 indicators for various user information and process surveillance purposes.

The slide rack assembly 6 may be shown in fig. 6. The slide rack assembly 6 may include a slide rack 61 which may have a capacity of eight slides 7 in individual slide receiving compartments 68, as shown in fig. 6. In connection with each compartment
10 68, a slide holder 62 may be provided. The slide holders 62 may include pivoting means or more broadly a vertical slide positioner which may include slide clips 69 and which may be pivotable between a horizontal slide position and a substantially vertical slide position and activation means 67. The slides 7, 7v may be individually pivotable in their slide holders 62, as the slide holder clips 69 may be pivoted by a
15 pushing of a nesting tab 67, of which two are provided, one for pivoting from a horizontal to a vertical position and one for returning the slide from a vertical to a horizontal position.

The slide rack may be in an upper position when the drawer 100 may be loaded with
20 one or more slides 7 and also during the staining process. After the slides 7 have been loaded, the slides 7 may be pivoted to a vertical position 7v and then the slide rack 6 may be lowered by the slide elevator 63, such that the vertically disposed slides 7v may be immersed into an underlying fluid containment element 101 or even a dip tank. The drawer assembly may also be shown in the figures 4, 5 and 7. The slide
25 elevator 63 may be adapted to agitate the slides 7v while they are immersed in the dip tank fluid.

The dip tank may be filled with a predetermined amount of a processing fluid from a transfer tank (see fig. 10) and may even be drained of fluid. The relevant processing
30 fluid may have been prior to that transferred from a supply tank to the transfer tank via pneumatic pressure means. In embodiments, the present invention includes supplying a processing liquid from at least one supply tank in a dip tank. By using a

transfer tank and controlling the fluid transfer by a pneumatic system including a vacuum pump, the pumping may be carried out without fluids coming into contact with pumping components. This may be advantageous since the risk of residues of fluids in the components may hereby be minimized.

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A total of eight drawers may be provided. Accordingly, this may include that eight dip tanks may also be provided in the apparatus. Each fluid containment element 101 or even a dip tank may accommodate up to eight immersed slides 7v at the time. A primary function of the dip tank may be to heat the fluid in the tank up to a
10 predetermined temperature, e.g. up to about 120°C from ambient temperature in a certain amount of time, e.g. about 15 minutes and maintain the predetermined temperature, e.g. about 120°C without any sign of boiling for a pretreatment processing time, e.g. about 10 to about 20 minutes after the slides have been lowered into the dip tank. After this process time may be passed, the heat may be turned off
15 and the slides 7v may be removed by raising the slide rack assembly 6 and thereby lifting the vertical slides 7v out of the dip tank. In embodiments the present invention may include processing a tissue sample. The fluid containment element or even the dip tank may be used for deparaffinization, re-hydration, heat induced target retrieval, among other processes. A pretreatment process may include any process including
20 but not limited to deparaffinization, re-hydration, heat induced target retrieval, and the like processes. These processes may be performed onboard the apparatus with the slides in a vertical orientation, immersed in individual tanks that can be filled with and emptied of various required reagents. For the target retrieval process, the fluid level in the dip tank may raise onto the label on the slide. The heating member may be
25 adapted to heat up and maintain a temperature of approximately about 95°C for a period of up to about 40 to about 60 minutes. The pretreatment process carried out in a fluid containment element or even the dip tank, may involve immersing the slides in a series of fluids for short periods of time, e.g. about 5 to about 10 minutes. The process of deparaffinization may be intended to first remove from the tissue sample
30 the paraffin in which it may be mounted, then remove the paraffin solvent, and then through a series of reagents progressively re-hydrate the sample.

As shown in e.g. figures 8 and 9, the fluid containment element 101 or even a dip tank may be elongated with an opening slot 102 through which the slides 7v may be inserted. This may result in a relative small dip tank volume, which in turn may allow for relatively rapid heating of the fluid in the tank and/or relatively low power consumption for heating up and maintaining the temperature of the fluid in the tank. The tank 101 may be filled and drained via a fluid connection tube 103 and the heating member 104 may be located in the lower section of the tank. The fluid containment element 101 or even a dip tank may be provided with insulating sidewall members on both sides to accelerate the heating thereby decreasing the heating times. The dip tank 101 may also be provided with sensor means (not shown) for registering the fluid level in the tank and a sensor for registering the temperature of the fluid, and feeding these data to the control system of the apparatus.

The pretreatment fluids or reagents may be stored in a number of individual containers, where some containers store fluids that are dedicated for deparaffinization, some for target retrieval and containers with 100% alcohol, distilled water and buffers. The containers may be advantageously provided with different volumes corresponding to the required amounts of the specific fluids for the performance of the pretreatment processes on the apparatus.

The fluid transfer between the supply containers 111 and the dip tanks 101 may be via a transfer tank 110, as shown in fig. 10. In embodiments, the present invention may include a fluid transfer element configured to transfer fluid between a dip tank and a supply tank with a transfer tank. The fluid transfer may be accomplished through positive and negative air pressure applied to the transfer tank 110 which may even be accomplished by a pneumatic pressure control adapted to apply positive respective negative air pressure to a transfer tank. Two separate, dedicated transfer tanks (not shown) may be provided, one for aqueous solutions and a second for organic solutions. Similarly, for emptying the dip tanks, the waste fluid may be transferred via the transfer tank 110 to the waste containers 112. Dedicated waste tanks may be provided, e.g. one for hazardous waste fluids and one for non-hazardous waste fluids.

Fluids may be transferred in both directions between any container and any fluid containment element or dip tank. The operational sequence of the fluid transfers may be determined by the control system of the apparatus. The deparaffinization reagents may be reused and periodically cycled from clean to dirty. Used dirty
5 deparaffinization fluids and dip tank rinse fluids may be discarded by the user or by the control system as hazardous waste. Target retrieval buffer and water are labelled “single use” fluids in the control system and transferred to waste after use.

Above, the apparatus and the method according to the invention are described
10 according to some explanatory embodiments. However, it is realized by the invention that many other variations and equivalents of the method and the apparatus may be carried out without departing from the scope of the invention as specified in the accompanying claims.

15 As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both tissue sample processing and staining techniques as well as devices to accomplish the appropriate processed and stained tissue samples. In this application, the tissue sample processing and staining techniques are disclosed as part of the results shown to be achieved by
20 the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed
25 by this disclosure.

The discussion included in this application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain
30 the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this

disclosure. Where the invention is described in device-oriented terminology, each element of the device implicitly performs a function. Apparatus claims may not only be included for the device described, but also method or process claims may be included to address the functions the invention and each element performs. Neither
5 the description nor the terminology is intended to limit the scope of the claims that will be included in any subsequent patent application.

It should also be understood that a variety of changes may be made without departing from the essence of the invention. Such changes are also implicitly included in the
10 description. They still fall within the scope of this invention. A broad disclosure encompassing both the explicit embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for any subsequent patent application. It should be understood that such language
15 changes and broader or more detailed claiming may be accomplished at a later date. With this understanding, the reader should be aware that this disclosure is to be understood to support any subsequently filed patent application that may seek examination of as broad a base of claims as deemed within the applicant's right and may be designed to yield a patent covering numerous aspects of the invention both
20 independently and as an overall system.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. Additionally, when used, the term "element" is to be understood as encompassing individual as well as plural structures that may or may
25 not be physically connected. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus
30 terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be

substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "slide immerser" should be understood to encompass disclosure of the act of "immersing" -- whether explicitly discussed or not -- and, conversely, were there effectively disclosure of the act of "immersing", such a disclosure should be understood to encompass disclosure of a "immerser" and even a "means for immersing" Such changes and alternative terms are to be understood to be explicitly included in the description.

Any patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. Finally, all references listed in the list of Information Disclosure Statement by Applicant or other information statement filed with the application are hereby appended and hereby incorporated by reference, however, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

Thus, the applicant(s) should be understood to have support to claim and make a statement of invention to at least: i) each of the tissue sample processing and staining devices as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and

methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) each system, method, and element shown or described as now applied to any specific field or devices mentioned, x) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, xi) the various combinations and permutations of each of the elements disclosed, and xii) each potentially dependent claim or concept as a dependency on each and every one of the independent claims or concepts presented.

With regard to claims whether now or later presented for examination, it should be understood that for practical reasons and so as to avoid great expansion of the examination burden, the applicant may at any time present only initial claims or perhaps only initial claims with only initial dependencies. Support should be understood to exist to the degree required under new matter laws -- including but not limited to European Patent Convention Article 123(2) and United States Patent Law 35 USC 132 or other such laws-- to permit the addition of any of the various dependencies or other elements presented under one independent claim or concept as dependencies or elements under any other independent claim or concept. In drafting any claims at any time whether in this application or in any subsequent application, it should also be understood that the applicant has intended to capture as full and broad a scope of coverage as legally available. To the extent that insubstantial substitutes are made, to the extent that the applicant did not in fact draft any claim so as to literally encompass any particular embodiment, and to the extent otherwise applicable, the applicant should not be understood to have in any way intended to or actually relinquished such coverage as the applicant simply may not have been able to anticipate all eventualities; one skilled in the art, should not be reasonably expected to have drafted a claim that would have literally encompassed such alternative embodiments.

Further, if or when used, the use of the transitional phrase “comprising” is used to maintain the “open-end” claims herein, according to traditional claim interpretation. Thus, unless the context requires otherwise, it should be understood that the term “comprise” or variations such as “comprises” or “comprising”, are intended to imply

5 the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible.

10 Finally, any claims set forth at any time are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of

15 the incorporated content of such claims or any element or component thereof from the description into the claims or vice-versa as necessary to define the matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or

20 treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.